

HEALTH HAZARDS
OF
MYCOTOXINS IN INDIA



National Institute of Nutrition, Hyderabad
INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI

1978

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IN INDIA

Ramesh V. Bhat

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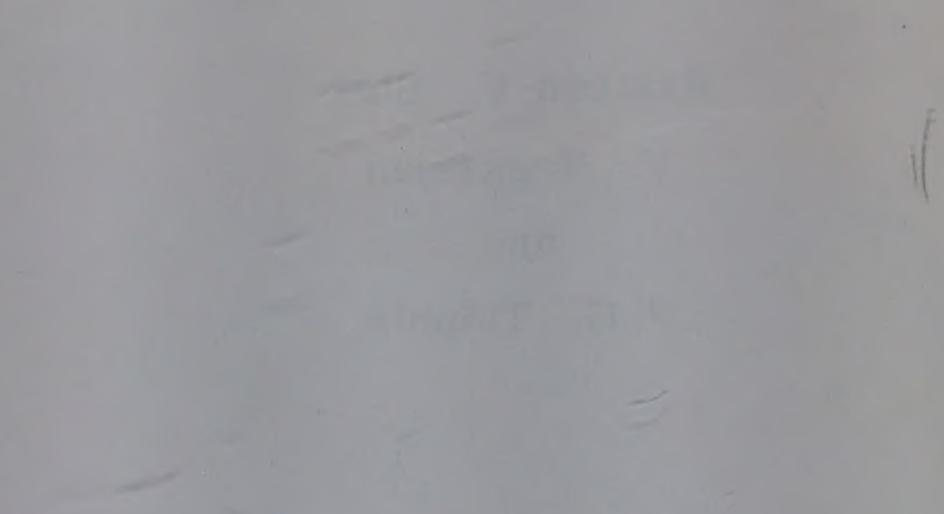
P. G. Tulpule

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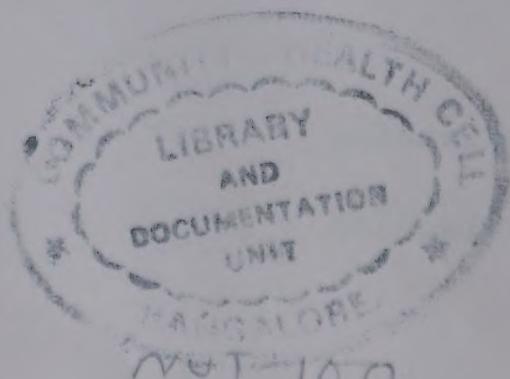


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FOREWORD

During the past two decades, it has become clear that mycotoxins can contaminate human and animal foods to an extent not realised before. Although knowledge of the health risks that may be associated with mycotoxic contamination of food and feeds is far from complete, considerable data have already become available and have prompted several organizations to expand their research efforts on mycotoxin research and control programmes.

The danger from mycotoxins is specially high in developing countries of the world because of climatic conditions which are favourable for mould growth, and inadequate pre and post-harvest practices which promote elaboration of toxins in staple food-grains. Also, widespread undernutrition in these countries may render the population more susceptible to hazards of mycotoxins.

It is appropriate that the National Institute of Nutrition, Hyderabad, which has done pioneering work in this field over the last decade, is bringing out this report which reflects current scientific knowledge arising from work carried out in this country. This comprehensive report will be useful for toxicologists, medical men, veterinarians, post-harvest technologists, food technologists and mycologists, both in this country and elsewhere. It is hoped that the awareness and concern generated in the scientific community in this field would be exploited for initiating national programmes for monitoring and surveillance of mycotoxins in foods which are of immediate relevance to health and welfare of man and livestock.

C. GOPALAN
Director-General
Indian Council of Medical Research
New Delhi.

ACKNOWLEDGEMENT

Information contained in this report was prepared as a country report on mycotoxins for the Food and Agriculture Organization in connection with the United Nations Environmental Programme.

Grateful thanks are due to Dr. S.G. Srikantia, Director, National Institute of Nutrition, Hyderabad, for his keen interest in the compilation of the report. Thanks are also due to Dr. K.O. Herz and Dr. R.K. Malik of Food and Agriculture Organization of the United Nations, Rome, Italy, for their valuable cooperation.

INTRODUCTION

Food Science and Consumer Protection Group of the Food Policy and Nutrition Division of the Food and Agriculture Organisation of the United Nations, Rome, suggested that a country status report on mycotoxins be prepared covering all aspects of mycotoxins in India. An attempt has been made here to bring under one cover all relevant information on the problem of mycotoxin contamination of foods and feeds. The material presented has been compiled on the basis of all available published literature on the subject, authentic reviews, reports of research projects on mycotoxins supported by US-PL-480 funding or other national agencies. Besides these, useful information has been collected by a standard questionnaire proforma, circulated to all agricultural universities, Veterinary Research Centres, Directors of Animal Husbandry of all the states, Government and Private owned poultry and dairy projects, commercial concerns dealing with bulk manufacture and distribution of animal feeds, national agencies like Indian Council of Agriculture Research, Indian Council of Medical Research, Council of Scientific and Industrial Research, Food Corporation of India, Central Committee for Food Standards, Indian Standards Institution, agencies concerned with feeding programmes such as UNICEF, CARE, UNDP, Indian Red Cross Society etc.

This country report has attempted to highlight the following major aspects of the problems concerned with mycotoxin contamination in foods and feeds:-

- (i) General conditions leading to fungal contamination;
- (ii) Occurrence and prevalence of fungal contamination;
- (iii) Mycotoxicoses in animals;
- (iv) Mycotoxicoses in human;
- (v) Approaches towards prevention and control;
- (vi) Centres with research capability for study of mycotoxins;

- (vii) National regulations for tolerance limits; and
- (viii) The need for a national programme of surveillance for mycotoxins in foods and feeds.

As the report indicates, there has been a growing concern about the problem of aflatoxins and in view of the known health hazards of these, work of aflatoxins have naturally received great impetus. This concern and awareness could very well be exploited for initiating a national programme for monitoring and surveillance of mycotoxins which are of immediate relevance to the health and welfare of live-stock and man.

Grateful thanks are due to the Food and Agriculture Organisation, Rome for providing the opportunity to prepare this country report and to Dr. K.O. Herz of F.A.O. for active co-operation.

1. GENERAL CONDITIONS LEADING TO FUNGAL CONTAMINATION

Tropical conditions in India, harvesting practices, post-harvest storage practices, high temperature, high moisture levels during the monsoon season, unseasonal rains and sudden floods damaging standing crops and stored food grains -- all these appear to be conducive for fungal invasion, proliferation, contamination and elaboration of mycotoxins. Paddy, one of the major staples in many parts, is often harvested with high moisture content. Improper drying of paddy after parboiling process is yet another risk. Peanuts immediately after lifting from the ground at harvest-time invariably contain high moisture. Drying of these peanuts of the summer crop does not pose many problems but conditions for drying of the winter crop are often not favourable due to monsoons. It becomes obvious therefore that there appear to be high-mycotoxin-risk crops combined with high-mycotoxin-risk climate and high mycotoxin-risk storage practices - all of which combine to create an extremely high mycotoxin hazard in live-stock and human population.

The storage structures could be classified into three major categories as under-ground, above-ground and roof storage. The under-ground structures are often made of mud, mud-plastered with cow dung or masonry work and food grains are heaped inside and closed with mud-plaster or wooden plates and covered with dry-hay. There are several types of storage structures of the above-ground group. These are structures of earthen-ware, bamboo covered with rope and straw; stone-slab bins etc. and are called differently in different regions as "gudu; kanaja; panatha; mudi; gade; gadebutta; gummi; bandijella; garise; moola, puri; pathera; arugupatora; boremu; kotlu" etc. In parts of Rajasthan and Himachal Pradesh, maize cobs are stored on roof tops tied to ropes. This type of storage is usually exposed to rain. In the urban areas, the most commonly used structures are earthenware pots, metal-bins or hundas or gunny bags. In most of the store-houses owned by the state, Food Corporation of India or Private Whole-sale Food grain Dealers, gunny bags storage in well-built godowns is practised. In these, gunny bags are piled up one over the other on raised platforms. There are a few centres where bulk storage is done in silos. Recently, the Food Corporation of India have initiated a

few centres where bulk open-storage is practised with protective polythene sheets overhead.

In the most commonly employed storage structures, the high moisture of the kernels of the food grains at focal points become susceptible to fungal attack. The other important factors appear to be the susceptibility to insect infestation which in turn increases susceptibility to fungal contamination. Work done at the Central Food Technological Research Institute, Mysore revealed certain insects do transmit spores of fungi, particularly those of *A. flavus* to stored grains. From ten species of insects commonly infesting food grains, fungal isolates of *A. flavus*, *A. ochraceus* and *Penicillium islandicum* were isolated and found to be toxigenic (Srinath et al, 1971; 1973). A more recent study has shown that rice weevil, the most common pest in rice, sorghum and wheat, under conditions of natural infestation in these stored foods showed the presence of fungi, the most predominant being *A. flavus* (Raghunathan et al. 1974).

2. OCCURRENCE

Several studies have been conducted in different parts of the country to examine the occurrence of fungal contamination in various agricultural commodities (Fig. 1). These studies appear to have three major objectives viz.

- (i) Identification of the predominant mycoflora in terms of the genus and species of fungi involved
- (ii) Toxigenic potential of the fungal isolates and
- (iii) The nature of mycotoxins produced. Such studies also served to assess the incidence of fungal contamination in different types of commodities. The available reports on the occurrence of fungal contamination have been grouped into the following categories for convenience.

These relate to:

- (i) Oil seeds and their products
- (ii) Cereals and millets
- (iii) Multi-commodity prevalence and
- (iv) Toxigenicity of the fungal isolates.

2.1 OIL SEEDS AND THEIR PRODUCTS:

In one of the earliest studies conducted by the National Institute of Nutrition, it was found that in the groundnut samples screened, about 9 per cent of the samples, *A. flavus* could be detected and 12 per cent of the samples collected had aflatoxin levels ranging between 1 to 5 ppm. The region particularly selected for this study was coastal districts of Andhra Pradesh where there is high humidity - a factor favourable for the growth of the fungus. The levels of contamination and the seasonal variations were assessed. Pods and cakes from the summer crop (rabi) and winter crop (kharif) were screened. A total of 298 pod-samples

and 92 cake samples of the rabi season, 445 pod-samples and 49 cake samples of the kharif season were screened. The levels of fungal contamination (*A. flavus*) and the levels of toxin were found to be invariably markedly higher in the kharif season (Rao et al, 1965). In another study, Sreenivasamurthy et al. (1965), assessed the incidence of aflatoxin contamination in peanut samples drawn from the city of Mysore and found that of about 150 isolates of *A. flavus* isolated from the groundnut samples, four were toxin-producing. Yet another survey in 1965-67 on peanuts and cakes drawn from three districts each of Gujarat, Andhra and Madras states, involving about 600 samples showed greater contamination in samples drawn from Andhra and Madras regions. About 20-40 per cent of the kernels and 82 per cent of the oil-cakes contained aflatoxin at fairly high levels (Anonymous, 1967). During a survey in 1967-68, nearly 50 per cent of the 500 samples of groundnuts from West coast contained aflatoxin B₁ with a range of 0.10 to 0.25 ppm (Wagle, 1970). A more recent study conducted at the Agricultural University at Jabalpur, found that out of about 97 samples of groundnut-cake mostly drawn from Madhya Pradesh region, more than 50 per cent of the samples were positive for aflatoxin (Anonymous, 1976c).

In a study of aflatoxin contamination in peanut oil, it was found that unrefined oil samples had an aflatoxin content of 0.02 to 0.2 ppm with a mean of 0.1 ppm. In oils obtained from peanuts stored for about six months, the range of toxin was 0.06 to 0.26 ppm with a mean of 0.14 ppm. No aflatoxin was detectable in refined oil samples (Dwarakanath et al, 1969, Basappa and Sreenivasamurthy, 1974). There appear to be practically no reports on the occurrence of fungal contamination, particularly of aflatoxins in other oil-seed crops such as sesame, mustard etc. The occurrence of aflatoxin was reported in Copra though no information on the levels of the toxin is available (Narasimhan, 1968).

A study with regard to cotton seed conducted during 1967-70, on 232 samples drawn from Maharashtra, Andhra and Gujarat, indicated that 93 per cent of the samples from humid areas and 68 per cent of the samples from dry areas were found to be infected with fungi, predominantly with *A. flavus*. In the samples from humid areas, 78 per cent contained aflatoxins and of these, 48 per cent showed toxin at levels higher than 500 ppb. Only 31 per cent of the samples from the dry areas contained toxins and 28 per cent of these did not exceed the toxin level of 50 ppb. There was a close correlation between the toxin content on the one hand and the degree of green-yellow fluorescence under ultraviolet light in the cotton seeds infected with *A. flavus*, on the other (Raghavendra Rao et al, 1970; Vedanayagam et al, 1971).

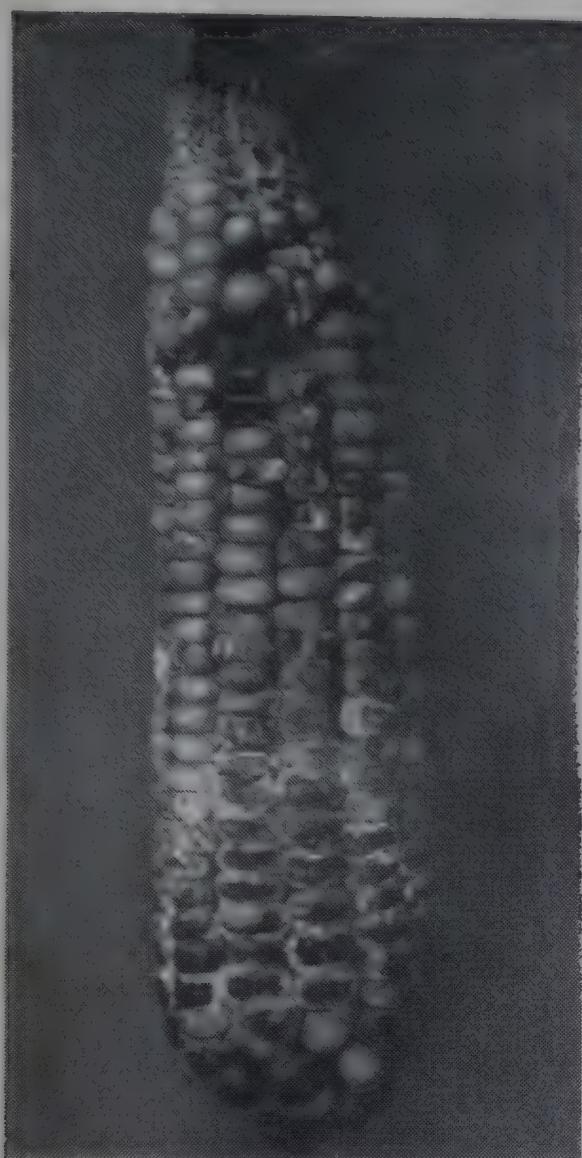


Fig. 1. Maize cob infected with fungi.

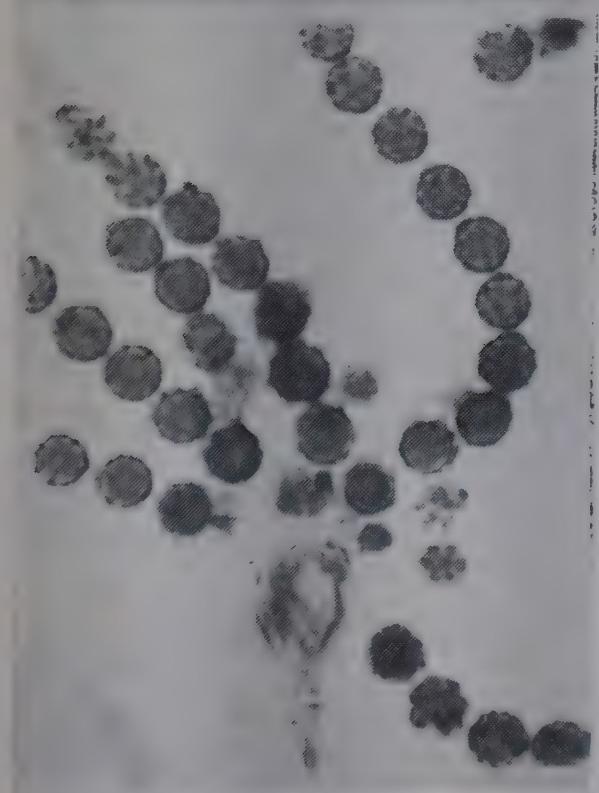


Fig. 2. Photomicrograph of one of the toxicogenic strains of *Aspergillus parasiticus* $\times 900$.

2.2 CEREALS AND MILLETS:

In a study in the coastal district of Karnataka state, covering 15 villages and a total population of about 1.5 lakhs, the fungal load in a representative cross sectional number of samples of rice was examined. Of these, 15 samples were positive for aflatoxin. However, there is no precise information about the levels of the toxin. The most commonly seen fungi were *A. flavus*, *A. candidus*, *Penicillium citrinum* (Sreenivasamurthy, 1975). A limited study of screening of "kuruvai" crop of paddy harvested with high moisture from Thanjavur district of Tamilnadu showed that parboiled rice prepared from this crop had traces of aflatoxin. Of the total of 18 samples screened, seven were positive and the toxin levels ranged between 30 to 130 ppb (Unpublished data, National Institute of Nutrition, Hyderabad, 1976). A screening investigation on head molds of sorghum grains in Pantnagar (U.P.), showed infection with *Curvularia*, *Fusarium*, *Aspergillus* and *Penicillium* species and aflatoxins B₁; B₂; G₁; and G₂ were detected. The species of *Aspergillus* in this study resembled *A. flavus* (Tripathy, 1973). In a survey in Western India in 1974, it was found that quite a number of samples maize grains and maize cobs were infected with *A. flavus* and the aflatoxin levels were appreciably high (6 to 15 ppm). One of the maize samples showed the presence of *A. parasiticus* (Krishnamachari et al, 1975b). An investigation on head models of jowar (*Sorghum vulgare*) from Hyderabad showed infestation with *Fusarium incarnatum*. Further analytical and toxicological studies revealed that the isolate is toxigenic and produces T₂ toxin (Anonymous, 1976a Rukmini & Bhat, 1978). An investigation in collaboration with the Indian Grain Storage Institute, Baptla (Andhra Pradesh), was carried out by the National Institute of Nutrition, Hyderabad, to examine the mycoflora of samples of paddy, sorghum and other millets from three regions in Andhra Pradesh. This study involved the screening of about 300 samples of paddy, 70 samples of jowar and 30 samples of ragi (*Eleusine coracana*) besides collection of all relevant background data regarding the type of storage structures, duration of storage etc. This study revealed the predominance of field fungi such as *Alternaria*, *Drechslera* and *Curvularia* and several non-sporulating forms in the paddy samples. In addition contamination with *Aspergillus* species was also found in a number of samples (Unpublished data, National Institute of Nutrition, Hyderabad, 1976).

2.3 MULTI-COMMODITY SCREENING:

During 1967-73, a screening project confined to the city of Madras, investigated the mycoflora of cereals like rice, wheat, maize and pulses like tur dal (*Cajanas cajans*), bengal gram

(*Cicer arietinum*) and mung (*Phaseolus radiatus*). Samples were drawn from some of the city warehouses and their mycoflora and toxicity tested. The general mycoflora detected included species of *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, *Helminthosporium*, *Stachybotrys* and *Pithomyces*. Some of these were found to be toxic on the basis of studies on rice-moth larvae, chicks, mice and rats. Citrinin from *Penicillium citrinum* isolated from contaminated foods was extracted, purified and toxicological properties studied (Anonymous, 1973).

A study of screening fungal contaminants in samples of cereals, millets, pulses and oil seeds was carried out in April-May, 1975 in parts of Uttar Pradesh. The samples for the study were collected from fields and purchase centres and included 190 of wheat, 50 of barley, 3 of oats, 16 of pigeon pea (*Cajanus cajan*), 13 of bengal gram (*Cicer arietinum*), 19 of white peas (*Pisum sativum*), 3 of soya beans, 16 of black mustard (*Brassica* species), 6 of peanuts, 4 of castor seeds and 9 of linseeds. The most commonly encountered fungal isolates includes species of *Aspergillus*, *Fusarium*, *Helminthosporium*, *Alternaria* and *Rhizopus*. *Alternaria* and *Aspergillus* species were the dominant contaminants. 91 isolates of *A. flavus* were tested for toxigenicity and majority of them were toxigenic. The biological toxicity of some of these have been tested on chicks or rats, on the basis of high mortality (Vora, 1976). Investigations on the mycoflora of poultry feeds and mouldy food grains revealed the presence of several toxigenic and non-toxigenic fungi (Katoch et al, 1975; Jhala, 1976).

Other agricultural commodities in which fungal contamination has been examined include Tapioca (*Manihot utilissma*), cash crops like cashew and some spices. A limited study on 20 samples of Tapioca obtained from Kerala state showed visible mold growth with *A. flavus*, *A. glaucus* and *A. niger* present along with *Penicillium* and *Rhizopus*. Further analysis of the samples showed a blue-fluorescent spot behaving like aflatoxin B₁ on thin-layer chromatograms. However, the material in biological tests was not toxic. This emphasises the importance of confirmatory tests to be performed on materials suspected of aflatoxin contamination (Nagarajan et al., 1973a). The problem of fungal contamination in other cash crops (Cashew and spices) has received only limited attention. A screening programme at the Central Food Technological Research Institute, Mysore has been investigating this aspect. Moulds commonly encountered in cashew and spices (Cumin, fenugreek, mustard, ajowan, fennel, black pepper, asafoetida etc.) include *A. niger*, *A. oryzae*, *Aspergillus* spp, *Penicillium* spp, *Trichoderma* spp and *Mucor* (Anonymous, 1971, 1972).

2.4 TOXIGENICITY OF THE FUNGAL ISOLATES:

A critical analysis of the reports available on the toxigenicity of fungal isolates of different genera and species, isolated from different agricultural commodities in India, indicates greater emphasis on the toxigenicity of *A. flavus* isolates than others. From available reports, there seems to be a predominance of *A. flavus* in India with only an occasional report on the occurrence of *A. parasiticus* (Fig. 2). This is of great significance, since generally *A. parasiticus* strains produce much higher levels of toxin than do strains of *A. flavus* (Nagarajan and Bhat, 1973). Most of the isolates of *A. flavus* seen in India are toxigenic and predominantly produce aflatoxins B_1 and B_2 in moderate amounts only. A study by Maggon *et al* (1969) on seven strains of *A. flavus* from the soils in Delhi indicated that all of them produced only aflatoxins B_1 and B_2 . Kang (1970) reported that all the aflatoxin producing isolates of *A. flavus* elaborated only B_1 . The studies of Rao *et al* (1965) on about 100 isolates of *A. flavus* from groundnuts, that of Raghavendra Rao *et al* (1970) and Indulkar *et al* (1971) on about 2533 isolates of *A. flavus* isolated from cotton seeds produced aflatoxin B_1 and B_2 and a majority of the isolates were toxigenic. Only one isolate was found to produce B_1 , B_2 , G_1 and G_2 . Of the 21 isolates of *A. flavus* obtained from cotton maize and wheat, only 16 were found to produce B_1 , as confirmed by bioassay of Okra seedling (Mehan and Chohan, 1973). Subramanyam and Rao (1974a) found that out of 240 isolates only 72 were toxigenic. These workers also reported the occurrence of aflatoxin and citrinin in groundnuts (Subramanyam and Rao, 1974b). This group also found that aflatoxin production to be more on natural substrates like groundnut, copra and castor than on sunflower, safflower, niger and sesame.

The toxigenicity of species other than that of *A. flavus* have been studied only to a limited extent. The studies at the University Biochemical Laboratories at Madras have produced evidence of the toxigenicity of *A. candidus*, *Penicillium citrinum*, *P. oxalicum* and *P. piceum* (Anonymous, 1973). The investigations at the Central Drug Research Institute have resulted in evidence of positive toxigenicity of *Trichoderma* spp. *A. nidulans*, *A. niger*, *A. ochraceus*, *A. tamari* and *Fusarium* spp (Vora, 1976). There is an isolated report that *A. tamari* produced aflatoxins in groundnuts (Lalitha Kumari *et al*, 1970).

3. MYCOTOXINS IN ANIMALS

In India, prior to 1960, outbreak of mycotoxicosis in animals did not receive much attention. Despite the worldwide awareness of harmful effects of mycotoxins, reports on the occurrence of mycotoxins in animals feeds and the diseases caused by them in animals in India are a few. This may be mainly due to the lack of facilities and expertise in detection of mycotoxin rather than the absence of them in Indian material. It is generally acknowledged that damaged and mouldy food grains, once rejected as unfit for human consumption, enter animal feeds since there is as yet no strict quality control with respect to mycotoxins in animal feeds.

3.1 AFLATOXICOSES IN DAIRY CATTLE:

One of the earliest outbreak of aflatoxicosis in Cattle was recorded by Sastry *et al* in 1965. A disease characterised by loss of appetite, diarrhoea, dullness, ascites, emaciation, anaemia and icterus involved 24 murrah buffaloes in Andhra Pradesh. Based on the clinical course, autopsy findings and histological studies of the livers of the animals that died, it was speculated that the toxin was present in certain consignments of groundnut cake fed to these animals. The histopathological features included centrilobular hepatic cell necrosis, bile duct proliferation and central vein occlusion. During the years 1966-1968, a number of buffalo-calves and young crossbred white cattle died with severe liver damage in the district livestock farm at Kodappanakunnu, Kerala State. High levels of aflatoxin were found in the animal rations (C.G. Sivadas, Personal Communication). Another suspected aflatoxin poisoning in a she-buffalo causing abortion was reported recently. This was based on histopathological changes in the liver of the aborted foetus (Mohiuddin and Ali, 1973). An outbreak of aflatoxicosis in dairy cattle involving death of 58 crossbred cattle in a herd of 126 from Karnataka was reported by Gopal *et al* (1968). The symptoms included anorexia, loss of condition, apathy, depression, corneal opacity, ascites, diarrhoea and occasional nervous symptoms like, excitement and walking in circles. Groundnut cake was a major component in the feed mix and *Aspergillus flavus* was found in the feed mix. The level of aflatoxin in feeds ranged from 1.1 to 25.0 ppm.

3.2 AFLATOXICOSIS IN POULTRY:

Although India has a considerable poultry population, reported outbreaks of mycotoxicosis are a few. In 1965, heavy mortality occurred among ducklings in the Government duck farm at Niranam, Kerala. A study conducted by the Kerala Veterinary College and Research Institute revealed that the ration of these animals had "high levels" of aflatoxin and the pathological features were identical with those described for aflatoxicosis. The survivors of the acute attack went off laying and developed malignant hepatomas after a year (C.G. Sivadas, Personal Communication). Heavy mortality (precise figures of mortality/morbidity not available) among ducklings in Hosur cattle farm in Tamilnadu in 1962 was encountered (Chandrasekharan, Personal Communication). In a later study Chandrasekharan (1968) isolated toxigenic strains of *Aspergillus flavus* from the sample of groundnut cake received from District Livestock farm, Orthanad, Tanjore, Both from the groundnut meal and from cultures isolated, aflatoxin B₁, B₂ and traces of G₁ and G₂ were obtained. Pooled sample was estimated to contain 6.2 ppm of aflatoxin.

An outbreak of aflatoxicosis in fowls in Karnataka involved the death of 2219 chicks (Gopal *et al*, 1969). The symptoms were severe sudden anorexia, loss of weight, staggering gait and convulsive movements. Subacute and chronic cases showed petechial haemorrhage, enteritis, ascites and histopathological changes typical of aflatoxicosis, such as yellow liver, fatty changes and biliary epithelial hyperplasia.

3.3 AFLATOXICOSIS IN RABBITS:

Outbreaks of aflatoxicosis in other animals include those in rabbits, pigs and dogs. In a private farm in Kulu Valley 4000 rabbits died. In the 15 autopsies carried out in the animals which died of chronic toxicity the characteristic changes in the liver were marked proliferation of connective tissue and hyperplasia of the bile duct. The animals were fed with pelleted diet and toxicological examination of these pellets were positive for aflatoxins. The symptoms observed were prolonged weakness and "hide bound" condition (Mehrotra and Khanna, 1973).

3.4 AFLATOXICOSIS IN PIGS:

Natural occurrence of aflatoxicosis in pigs were reported from Pig Breeding Farm at Mannuthy, Kerala. The affected animals were 'stunted' and at autopsy, the liver revealed considerable liver damage. Aflatoxin contaminated groundnut cake was incriminated to have caused the liver damage. Feed samples from various

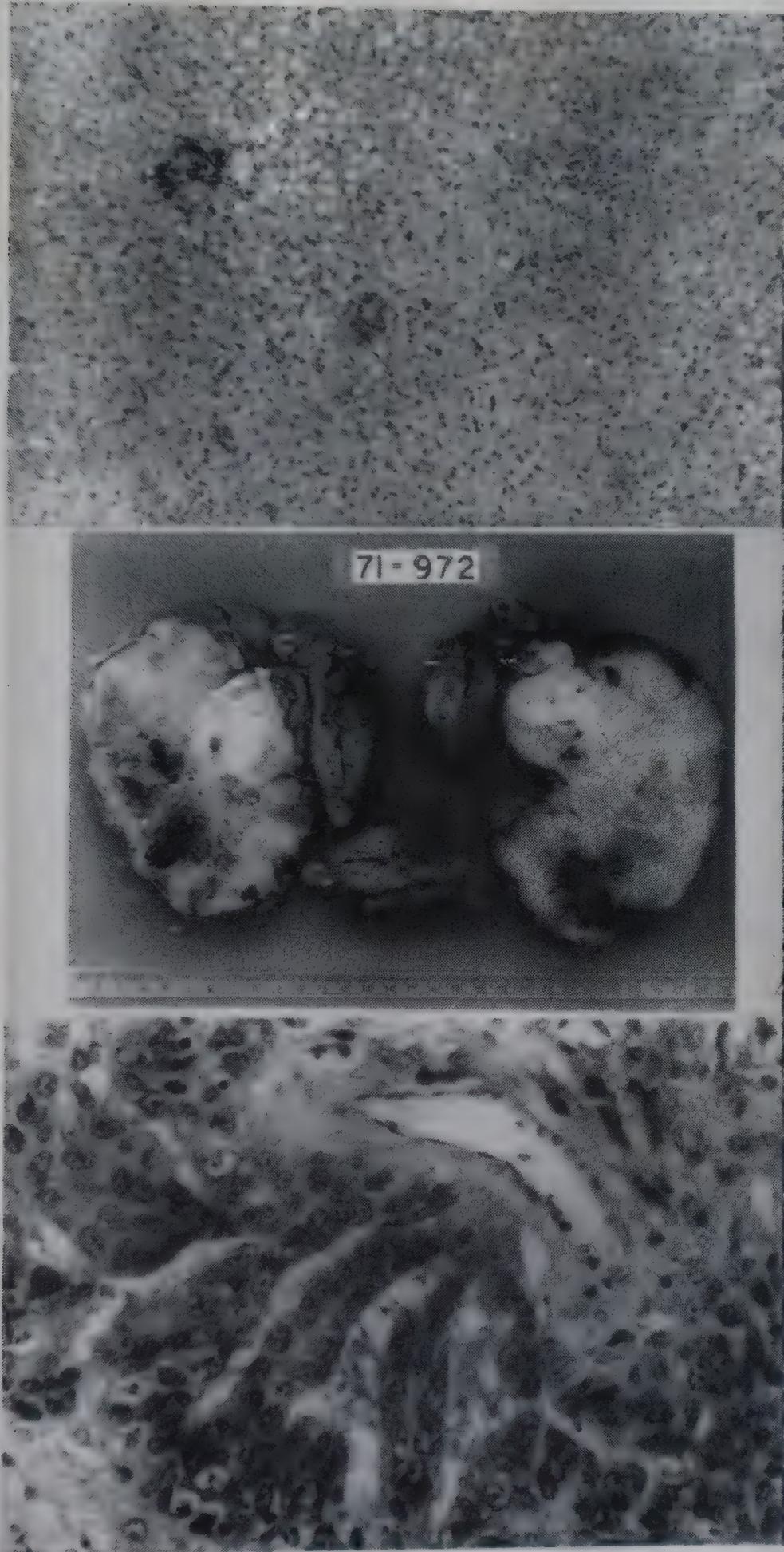
livestock farms like Thumburumuzhi, Mannuthy and Thiruvazham Kunnu revealed that some items in all farms were contaminated with aflatoxin. The aflatoxin levels in samples responsible for causing disease in animals in all these cases is estimated to be generally above 20 ppm (C.G. Sivadas, Personal Communication).

3.5 AFLATOXICOSIS IN DOGS:

During an epidemiological study on the outbreak of aflatoxicosis in humans in western India, it was found that invariably in all the households where humans were affected, dogs were also affected and most of them died. Invariably, tribal people in these areas, who were the worst affected tend a dog in each household. The symptoms in dogs were anorexia in the early stages and rapidly developing ascites later resulting in high mortality. The dogs were subsisting on a diet left by the humans and humans were consuming preparations made out of maize, contaminated with aflatoxins. Levels of toxin ranged between 6.5 and 15.5 ppm. (Krishnamachari *et al*, 1975a,b,c).

3.6 AFLATOXICOSIS IN EXPERIMENTAL ANIMALS:

The toxic effects of aflatoxins have been documented in several species of laboratory animals in different parts of the world. The effect of aflatoxins on primates, was, however, demonstrated for the first time by the National Institute of Nutrition, Hyderabad. Monkeys receiving as little as 0.5 mg of toxin for 3-4 weeks developed fatty liver (Fig. 3) (Tulpule *et al*, 1964). The incidence of chronic liver diseases is high in parts of the world where malnutrition is widespread. In view of this association, studies on the effect of protein malnutrition on the toxic effects of aflatoxin on the liver assume considerable importance. Studies on monkeys fed different levels of protein and exposed to aflatoxin, demonstrated that liver damage occurs more rapidly, and is of a greater magnitude in low protein groups than in adequate group (Madhavan *et al*, 1965). Similarly, short term experiments in rats have indicated that aflatoxin injury was markedly enhanced by feeding low protein diets (Madhavan and Gopalan, 1965). Moderate to severe lesions such as necrosis, bile duct proliferation and fatty changes were present in the livers in contrast to the controls which showed changes suggestive of a possible mild precancerous state. A subsequent study by the same group of workers on the effect of dietary protein levels on the carcinogenic action of aflatoxin in the rat revealed that low levels of dietary protein had an inhibitory effect on carcinogenesis due to aflatoxin in the rat (Madhavan and Gopalan, 1968).



- Fig. 3. Liver of monkey fed with aflatoxin showing severe fatty change and biliary fibrosis Haematoxylin & Eosin \times 90.
- Fig. 4. Cut surface of the hepatic tumour from an aflatoxin treated monkey. Necrotic and haemorrhagic areas appear greyish black.
- Fig. 5. Trabecular pattern of the growth by well differentiated neoplastic liver cells of the aflatoxin treated monkey. The trabeculae are bilaminar and enclose sinusoidal spaces. Note close resemblance with the structure of the liver. Haematoxylin \times 250.



A number of reports on the natural occurrence of neoplasia and cholangiocellular carcinoma in animals such as hen, ducks, ewe, cow, dog are available from India (Bandopadhyaya *et al*, 1966; Charan *et al*, 1976; Christopher *et al*, 1968; Rao *et al*, 1964; Sivadas *et al*, 1962). However, in none of the above cases, evidence is available to definitely implicate mycotoxins.

Hepatocellular carcinoma of a giant cell type was induced in a male monkey by feeding aflatoxins for 5.5 years (Fig. 4 5). The animal was given 50 µg of mixed aflatoxin 5 days/weeks by i.p. injection and double this dose by the same route for the next 11 months. At the end of one year, the route of administration was changed to oral dosage and increased to 200 µg. The carcinoma was detected when the animal died 2.5 years after the toxin administration was discontinued (Gopalan *et al*, 1972). A female monkey of the same experimental group but receiving half the amount of toxin as that of the male developed cholangiocarcinoma 5.25 years after the toxin was withdrawn (Tilak, 1975).

Yet another interesting finding is the aggravation of the toxic effects of aflatoxins in vitamin A deficiency in rats. Albino rats maintained on a vitamin A free diet for nine weeks and given 3.5 mg/kg of aflatoxin in a single intraperitoneal dose showed increased susceptibility to the toxin as evidenced by rapid mortality and severe liver damage. Liver damage was minimal in female rats as compared to males (Reddy *et al*, 1973). This observation is of interest since vitamin A deficiency is usually associated with protein-calorie malnutrition in developing regions and it is such malnourished groups of children who are also exposed to the risk of aflatoxin contamination. The effect of prednisolone, a corticosteroid on aflatoxins liver injury was studied in weaning rats. In the presence of higher dose of prednisolone, there was marked inhibition of bile duct proliferation and fat accumulation. The clinical signs and mortality due to the toxin did not appear to be influenced by the steroid (Madhavan, 1967). Cold exposure at 4°C in rats appeared to provide protection against acute liver damage by single administration of the toxin in doses appropriate for the body weight. Control animals maintained at 22°C administered similar dose showed marked characteristic changes in the liver (Tilak *et al*, 1976).

Studies on aflatoxin toxicity in ducklings revealed that in addition to the characteristic lesions such as bile duct proliferation, hepatic infarction occurs (Madhavan and Rao, 1966). In guinea pigs fed with various levels of aflatoxins, renal changes such as tubular epithelial reflux have been noticed (Madhavan and Rao, 1967). Tilak and Krishnamurthy (1973) studied the effect of

aflatoxin B₁ on liver regeneration following partial hepatectomy in rats. The results indicated that aflatoxin B₁ completely inhibited the increment in liver weight and markedly suppressed hepatocellular replication.

3.7 VARIATION IN SUSCEPTIBILITY:

In an attempt to explain the differences in susceptibility to aflatoxin toxicity in farm animals, the metabolism of toxin in the liver of cows, she-buffaloes, sheep and goats were studied (Yadgiri and Tulpule, 1975). It was observed that sheep and goat livers metabolised the toxin more efficiently and hence are relatively more resistant to the effects of aflatoxin than are cows and buffaloes.

An investigation was conducted to determine the extent of toxin contamination in milk produced by the cattle in Andhra Pradesh region. This study revealed that aflatoxin M₁ in amounts ranging from traces to 4.8 µg/l was present when the murrah she-buffaloes consumed groundnut cake contaminated with aflatoxins ranging from traces to 3 mg/kg (Yadgiri and Tulpule, 1974). This investigation revealed, that besides causing health hazards to animals, aflatoxin contamination can also pose danger to human health, if strict quality control is not maintained in animal feeds.

A comparative study of aflatoxicosis in crossbred jersey calves and buffalo calves showed that buffalo calves were more susceptible to aflatoxicosis. Clinically, icterus was present in both species. Weight loss, failure to thrive, weakness, emaciation and ascites were also noticed. Appreciable reduction in erythrocyte sedimentation rate was observed in buffalo calves. Liver was pale yellow, enlarged and fixable with round border (Rajan et al, 1973).

Rajan and Mohiyuddin (1973) studied the pathological changes in experimental aflatoxicosis in dogs, guinea pigs, ducklings and chicks with special reference to lesions in the pituitary, thyroid and adrenal glands. They observed proliferation of basophils and degeneration of acidophils in the pituitary gland. Hyperplastic and colloid type changes were observed in chicks and guinea pigs. Varying degrees of congestion and haemorrhage and proliferation of cells in zona fasciculata were commonly present in the adrenal gland in all species, but was most severe in chicks.

Besides buffaloes and cows, goat was also found to be susceptible to aflatoxicosis. Young and pregnant animals were

highly susceptible. Aflatoxins were secreted in the milk. Pathological changes included renal and hepatic degeneration and necrosis. These changes were noticed in the pituitary, thyroid and adrenal glands in the aflatoxin fed goats. Considerable reduction in serum vitamin A levels was also seen (Maryamma, 1973; Maryamma and Sivadas, 1973, 1975). Experimental studies with chicks indicated that aflatoxin impaired the digestive function and produced pathological changes in the intestine besides increasing the predisposition of chicks to coccidiosis (Mony, 1971).

3.8 MYCOTOXICOSES - OTHER THAN DUE TO AFLATOXIN:

Reports of mycotoxicosis in animals caused by other mycotoxins are few. Sporadic reports of outbreaks of diseases in animals like buffaloes due to some unknown aetiology have been suspected to have been caused by mycotoxins. In 1939, Shirlaw reported the occurrence of "Degnala" disease in buffaloes in Punjab of undivided India. The disease was characterised by malice, fever, pain in the abdomen, painful gait and anorexia. The occurrence of the disease in these areas was reported from time to time (Irfan, 1971). A peculiar disease similar to the "Degnala" disease afflicting 1400 buffaloes in Punjab occurred during 1969-1970. The disease was characterised by necrosis of the tips of the ear, tail and tongue and swelling of the extremities with subsequent peeling of the skin, leaving open wounds. In severely affected cases, the hooves and phalanges were cast off, somewhat resembling the classical ergot poisoning (Kwatra and Singh, 1971). Subsequently 'Degnala' disease was produced in buffalo calves by feeding IR 8 variety of paddy during winter, but not in summer. These paddy straws were found to be infected with moulds like *Aspergillus terreus*, *A. ficuum*, *A. versicolor*, *Absidia* and *Fusarium equiseti* (Kwatra and Singh, 1973). The disease again broke out in serious proportions in herds of buffalo and cattle in Punjab and Haryana. The symptoms were mainly necrosis and gangrene of the extremities (Fig. 6). The disease mainly affected buffaloes fed with paddy straw and was seasonal occurring in the cold season. The morbidity and mortality rates were 60 per cent and 22 per cent respectively in buffaloes and 14 per cent and 3 per cent respectively in cattle. From the rice straw, *Fusarium equiseti* and *F. moniliforme* were isolated. Of these, *F. equiseti* was found to be toxic by the rabbit skin bioassay method. Although no mycotoxin was identified, it was suspected that the disease may be due to a butenolide. However, definite proof for this was not forthcoming (Kalra et al, 1972; 1973).

Tripathy et al (1967) reported the death of 22 birds out of a flock of 236 Rhode Island red pullets in Orissa State poultry

breeding farm. The symptoms and post-mortem findings were similar to the "haemorrhagic syndrome" and "mycotoxicosis in poultry reported from other countries". Haemorrhagic syndrome in poultry with variable haemorrhages in subcutaneous tissue, muscle, liver, heart, spleen and kidney was also reported (Sharma and Singh, 1971). A disease diagnosed as Stachybotryotoxicoses characterized by diarrhoea with tenesmus, ruminal atony involving 160 cattle with a mortality of 40 per cent was attributed to the presence of *Stachybotrys atra* in straw samples (Rajendran *et al*, 1975).

4. MYCOTOXICOSES IN HUMAN

4.1 ERGOTISM:

The disease attributable to the consumption of mould damaged foods is not well documented in India. Ergotism which has been recorded in Europe during medival years was also not known to occur in India till the recent past (Bove, 1970). Ergotism due to the consumption of pearl millet infected with ergot (Fig. 7) was noticed during 1956 (Patel *et al.* 1958). Since then, sporadic reports of ergot poisoning in humans have been reported from different parts of the country especially from Maharashtra, Gujarat, Rajasthan and Karnataka. In India, the clinical manifestations of ergot poisoning through pearl millet, include nausea, vomiting, giddiness and somnolence. An investigation into the outbreak of ergotism in humans in Sikar and Jaipur districts of Rajasthan revealed that if the level of contamination of ergot exceeds 1.5 per cent, (w/w) clinical symptoms become evident. Even animals like camels, if fed the ergoty pearl millet are known to suffer from the disease (Krishnamachari and Bhat, 1976).

Bhide and Sheth (1957) conducted chemical and pharmacological studies on ergot alkaloids of pearl millet and concluded that they contain ergot alkaloids. Patel and Boman (1960) also studied the nature of ergot alkaloids of bajra and found them to be similar to ergot alkaloids of rye. Three alkaloids viz. ergometrine, ergotamine and ergokryptine were detected by Kannaiyan *et al* (1971) in ergot of pearl millet. However, the differences between the clinical symptoms in humans seen in India and that of the convulsive and gangrenous type seen in Europe, necessitated a reappraisal of the nature of alkaloids in ergoty pearl millet in India. These studies revealed that ergot of pearl millet contain alkaloids of the clavine group consisting mainly of agroclavine and traces of elymoclavine, chanoclavine, penniclavine and setoclavine. In contrast, alkaloids of ergoty rye and wheat mainly belong to the classical ergotoxine - ergotamine, ergometrine group. This was confirmed by the observation that ergot of rye and wheat, on hydrolysis yield various amino acids, whereas, the ergot alkaloids

of pearl millet do not leave any amino acid residues. The chemical differences were confirmed by biological experiments (Bhat et al, 1975, 1976). The infecting agent has also been identified as *Claviceps fusiformis* (Bhat, 1977). Singh and Hussain (1976) also found that ergot of bajra contains various clavine alkaloids and that the total alkaloid content of sclerotia vary in different agro-climatic regions. Since the chemical symptoms in man, chemical nature and biological effects of alkaloids and the identity of ergot causing fungus are different, the ergotism due to pearl millet in India, be referred to as enteroergotism while the ergotism due to ergot of rye in Europe be referred to as vascular ergotism.

Feeding trials with ergoty pearl millet to guinea pigs at different levels did not show any change in their heart, kidney and reproductive organ although the liver and lungs were severely affected (Kannaiyan et al, 1971). Bhat and Roy (1976) succeeded in producing experimental ergotism in monkeys by intraperitoneal injections of alkaloids of ergot of pearl millet (Fig. 8).

4.2 MOULDY RAGI POISONING:

During late 1920's, a mycotoxic disease in humans attributable to the consumption of finger millet "Ragi" (*Eleusine coracana*) was described from India. The main characteristics of the disease were "vomiting and purging". Persons consuming old ragi stored in "hagevs" suffered. The affected ragi was black in colour with pustular out growth in surface. The grains had greyish white covering and the fungus was identified as *Heterosporium* (Narasimhan, 1929).

4.3 KODO MILLET POISONING:

This is known to India since ancient times. Kodo millet (*Paspalum scrobiculatum*) is a minor millet grown in dry tracts of India and more than 3 lakh tonnes are produced in India. Kautilya's Arthashastra, a text written in 300 B.C. refers the poisoning of tigers by the grain (Shamasastri, 1960). The grains have often been reported to cause poisoning of man and animals when used as food. The grain and straw are reported to become poisonous only when the harvest is left in the field in rainy and wet conditions. A herd of 13 wild elephants which have eaten this crop were reported to have died. The main symptoms of Kodo poisoning are, unconsciousness, delirium with violent tremors of the voluntary muscles, vomiting and difficulty in swallowing (Anonymous, 1966). The symptoms observed within 20 minutes of taking the food were tremors, giddiness, excessive perspiration



Fig. 6. Affected feet of a buffalo calf, fed on toxic rice straw showing oedema and patchy necrosis of skin and separation of hoofs (Courtesy : Prof. D.S. Kalra, Haryana Agricultural University, Hissar).



Fig. 7. Ear head of bajra infected with ergot.



and inability to speak or swallow. The disease was not fatal and symptoms disappeared within 24 hours. Only a few of the samples were poisonous and a petroleum ether extract of the poisonous variety developed a red colour when shaken with concentrated sulphuric acid (Ayyar and Narayanaswamy, 1949). A lipid extract of this millet was found to cause tremors in dogs and monkeys. Crows were found to be susceptible with symptoms, such as vomiting, drooping of head, loss of power of movement and death (Ayyar and Narayanaswamy, 1948).

Kodo millet seeds *per se* were found to be harmless to animals but the kodo millet infested with fungi were found to be toxic (Iswarriah, 1951). Cases of poisoning of cattle by kodo millet were reported from different parts of India (Mia, 1960; Nayak and Misra, 1962). The isolation of a tranquilizing principle from the kodo millet has been claimed (Bhide and Aimeen, 1959). Various animals such as dogs, cats, monkeys, rats, mouse, guinea pigs, rabbit and pigeon were susceptible (Bhide, 1962). The symptoms were hyperexcitation, muscular incordination, spasms and staggering gait. Central nervous system depressants such as pentobarbitone, chloral hydras-magnesium sulphate combination and phenobarbitone were found to protect experimental animals from tremors and clonic convulsions (Gupta and Bhide, 1967). Experiments carried out in rats with fungus free kodo millet seeds indicated that they may be used profitably (Agarwal, 1964).

Recently Pendse (1974) and Pendse, *et al* (1974) examined a large number of samples of toxic grains from Maharashtra and found that the samples were always infested predominantly with *Phomopsis paspalli*. The ether extracts were toxic to dogs and showed symptoms typical of kodo toxicity. It would be interested to see whether from such samples of kodo millet which cause human toxicity, the cytochalasins could be isolated. From this fungus, two cytochalasins, kodo cytochalasin - 1, with LD 50 of 2 mg/kg and kodo cytochalasin - 2 with LD 50 of 5 mg/kg have been isolated (Patwardhan *et al*, 1974).

4.4 POLYURIA:

An epidemic form of a disease in human characterised by severe thirst, anorexia, weakness and polyuria with occasional symptoms of blurring vision, photophobia, palpitation, dyspnea on excretion, choking sensation, pain and cramps in the limbs and giddiness was traced to the consumption of pearl millet infected with the fungus *Rhizopus nigricans*. The disease was variously termed 'polyuria' and polydypsia syndrome, Poona disease, "Sasson Hospital syndrome". Three persons were reported to have died of

this disease (Deodhar *et al*, 1970). Though it has been demonstrated that rats fed mycelium of *Rhizopus nigricans* also had polydypsia and polyurea, no attempt was made to isolate the toxin (Narasimhan *et al*, 1967). Similar disease was reported in India by other workers (Deshmukh, 1953) Chandrachud *et al*, 1954 and Vishwanathan, 1954).

4.5 AFLATOXICOSIS:

During 1974, the National Institute of Nutrition, undertook a detailed investigation into an outbreak of acute hepatitis in tribal areas extending over 200 villages of Banswada district of Rajasthan and Panchmahal district of Gujarat. The disease was characterized by jaundice, rapidly developing ascites and portal hypertension (Fig. 9). About 500 persons predominantly males with a sex ratio of 2:1 in the age group of 5-14 and above 30 years were affected and more than 100 persons died of the disease. Maize (*Zea mays*) the staple food of this people in the area was found to be visible contaminated with *Aspergillus flavus* and aflatoxin levels ranged from 6.5 to 15.5 µg/gm. In an autopsied human liver, bile duct proliferation and multinucleate giant cells were noticed. Based on a combination of epidemiological, mycological, mycotoxic and histopathological studies, it was concluded that the disease was caused by the consumption of maize heavily contaminated with aflatoxins (Krishnamachari *et al*, 1975 a,b,c). An independent study conducted by the All India Institute of Medical Sciences also revealed that the disease is most likely to have been caused by the consumption of aflatoxin contaminated maize (Anonymous, 1975 a, Tandon *et al* 1977). The episode in western India implicating aflatoxin contamination in the causation of acute liver disease in man is perhaps the first report directly incriminating aflatoxin in foods as a health hazard. This episode also focussed attention on aflatoxin contamination in staples and emphasised the fact that even if the level of contamination are low, the risk can not be ruled out in view of the bulk-intake of staples for sustenance. A follow-up study by the National Institute of Nutrition indicated that the disease incidence and the level of contamination during late 1975 was markedly low (Bhat and Krishnamachari, 1977).

A study undertaken by the Central Food Technological Research Institute, Mysore in South Kanara has indicated that there is a positive correlation between aflatoxin content of foodgrains on the one hand and incidence of liver enlargement in children on the other. Those children who showed enlarged liver had abnormal red blood corpuscles in circulation (Sreenivasamurthy, 1975).

TABLE - 1

MYCOTOXICOSES AND MYCOTOXINS AFFECTING MAN AND ANIMALS

Mycotoxicosis	Fungal species	Commodity	Toxin	Species affected	System affected (or) symptoms	Location	Year
Aflatoxicosis	<i>Aspergillus flavus</i>	Maize	Aflatoxin	Man	Jaundice, ascites; liver damage and high mortality	Western India	1974
Polyuria	<i>Rhizopus nigricans</i>	Pearl millet	Unidentified	Man	Thirst, anorexia, polyuria	Poona	1953, 1954, 1967, 1970
Ergotism	<i>Claviceps fusiformis</i>	Pearl millet	Clavine group of alkaloids	Man	Nausea, vomiting, somnolence	Maharashtra, Rajasthan	1956, 1958, 1975
Mouldy Ragi poisoning	<i>Heterosporium</i>	Finger millet	Unidentified	Man	Vomiting, purging	Karnataka	1929
Kodo millet poisoning	<i>Phomopsis paspalli</i>	Kodo millet	Cytotochala-sins(?)	Man	Vomiting, unconsciousness, delirium, tremors.	Tamilnadu, Maharashtra	
Aflatoxicosis	<i>A. flavus</i>	Animal feeds (groundnut cake)	Aflatoxin	Dairy cattle	Anorexia, ascites, liver damage, abortion.	Andhra, Kerala Karnataka	1965, 1966, 1968
"do"		-do-	-do-	Poultry	Hepatoma, off-laying, ascites liver damage	Kerala, Tamil Nadu, Karnataka	1965, 1962, 1969
"do"		-do-	-do-	Rabbits	Liver damage	Himachal Pradesh	1973
"Degnala" Disease	<i>Fusarium</i> species		-do-	Pigs	Stunted growth, liver damage	Kerala	1968-69
Haemorrhagic syndrome	Not Identified	Maize	-do-	Dogs	Ascites, liver damage	Western India	1974
■ Stachybotryotoxicosis	<i>Stachybotrys atra</i>	Paddy straw	Butenolide(?)	Buffaloes	Anorexia, Necrosis of extremities	Punjab, Haryana	1939, 1971
		Animal feeds	Unidentified	Poultry	Haemorrhage in vital organs	Orissa, U.P.	1967, 1971
		Paddy straw	Unidentified	Dairy cattle	Diarrhoea with tenesmus	Tamilnadu	1973

Indian childhood cirrhosis (ICC), a serious disorder of liver in children is confined mostly to the Indian subcontinent. The aetiology of this disease is not clear and various hypothesis such as toxic, viral, hereditary factors have been put forward (Nayak *et al*, 1972). Robinson *et al* (1967) suggested that aflatoxins may have a role in the aetiology of ICC. According to Amla *et al* (1970, 1974) there is sufficient circumstantial evidence to show that children exposed to aflatoxin through breast milk and dietary items such as parboiled rice and unrefined groundnut oil may get Indian childhood cirrhosis. Aflatoxin B₁ in concentration ranging from 0.02 to 0.05 µg/24 hour collection of urine was detected in 7 per cent of the urine samples from cirrhotic children. In a subsequent communication Amla *et al* (1971) reported that malnourished children who had accidentally consumed aflatoxin contaminated (300 µg/kg) peanut protein flour developed hepatic lesions identical with that seen in Indian childhood cirrhosis. They also observed similar skeletal muscle changes in cirrhotic children, aflatoxin fed rats and children who consumed the aflatoxin containing meal (Amla *et al*, 1969, 1971).

Yadgiri *et al* (1970) observed the presence of compounds having some resemblance though not identical to aflatoxins in urine and liver extracts of children suffering from ICC. Contrary to this, Parpia *et al* (1972) confirmed the presence of aflatoxin in urine samples in 7 per cent of 332 children examined. However, aflatoxins are unlikely to be the cause of Indian childhood cirrhosis since this particular disease is absent in African countries where the incidence of fungal contamination in diets is fairly high (Nayak *et al*, 1972).

4.6 THE INCIDENCE OF HEPATOCELLULAR CARCINOMA IN INDIA:

The exact assessment of the incidence of cancer at various sites of the body is not available in India for want of registration of all cases of cancer, including liver cancer in the population. Incidence data based on mortality would be inaccurate for want of autopsy examination of such cases. Similarly, clinical data are of limited value except when histological examinations of biopsy or autopsy materials have been made.

Frequency rate of a neoplasm has often been expressed as per cent of all neoplasms at various sites of the body or per cent of all cases (neoplastic and non-neoplastic) available for autopsy during a specific period. In Visakhapatnam, primary liver cancer was 11.23 per cent of all malignant tumors and in Agra 0.67 per cent of all cases (Wahi, 1966) between 1960-62.

Percentage of primary liver cancer of all autopsies from different medical centres of India is as follows: Bombay 0.20 per cent (Gharpure, 1948); Bombay 0.38 per cent (Kshirsagar *et al*, 1968); Madras 1.60 per cent (Reddy and Rao, 1967); Vellore 1.74 per cent; Visakhapatnam 1.53 per cent; Guntur 1.10 per cent and Agra 1.20 per cent (Wahi, 1966).

The data suggests that atleast among the autopsy cases, primary liver cancer has not been infrequent in India. However, its relation to aflatoxin or other mycotoxins is not established. In this context, the observation on induction of hepatic carcinoma with aflatoxin in the rhesus monkeys is perhaps of great practical significance (Gopalan *et al*, 1972).

5. APPROACHES TOWARDS PREVENTION AND CONTROL

5.1 GENERAL APPROACHES FOR CONTROL OF MYCOFLORA:

As part of general measures for the control of microflora in stores grains, the well-known approach of the use of fungicides, fumigants and other chemical sprays has been under trial since long. A systematic study of the usefulness of this approach has been investigated (Majumder, 1974). These studies relate to the effectiveness of fungistatic agents as propionic acid and acetic acid; gaseous sterilents as methyl iodide, chloropicrin, ethylene bromide, ammonia and sulphur dioxide. Attempts made with luprosil (100 per cent propionic acid) showed that it prevents visible mold growth at 0.25 per cent level. The percentage of infection decreases as the dose of luprosil is increased from 0.25 to 0.4 per cent. Both luprosil (0.4 per cent) and acetic acid (0.2 per cent) were found to be effective in the treatment of sorghum in semi-large scale trials. Similar studies by Dhanaraj *et al* (1973) showed the effectiveness of luprosil in preventing fungal contamination in wet maize samples. Most of these treatments leave no residual effects on the stored grains.

5.2 AFLATOXIN CONTAMINATION:

There have been two major approaches for the prevention and control of the problem of aflatoxin contamination. The first approach has been that of separation of contaminated seeds (Fig. 10) or detoxification procedures either by removal of the toxin by extraction or by destruction of the toxin by suitable treatment. This approach has been under investigation at the Central Food Technological Research Institute, Mysore. Sreenivasamurthy *et al* (1965) reported that an aqueous solution 1 per cent NaHCO_3 extracted 100 per cent of the toxin as against 80 per cent removal by 1 per cent CaCl_2 solution. However, alkali treatment extracted 33 per cent protein as against only 6 per cent in CaCl_2 treatment. These trials have suggested that extraction of the material at neutral pH, using 1 per cent CaCl_2 could be usefully exploited. The alternative approach of the destruction of the toxin has also been under experimentation. A systematic study of detoxification

of peanut meal by hydrogen peroxide was reported by Sreenivasa-murthy *et al* (1967). In this method, treatment with 6 per cent H₂O₂ at 80°C for 30 min. resulted in destruction of the toxin to a level of 97 per cent. Duckling test of this processed meal indicated no toxic effects. Animal feeding tests showed that the protein quality of the treated meal is not altered significantly. Similar trials have also been done through ammonia treatment. Some of these methods have been found to be successful at the pilot-plant scale and appears to have been patented (Indian Patent No.12025 dated 16.3.1969). These methods have their limitations in that they need centralised processing units, quality control, etc. and tend to increase the cost of the end-product (Achaya, 1975).

Genetic approach resulting in resistance to the development of the fungus or the elaboration of the toxin has been under study for sometime. The development of commercially popular varieties that would resist the development of toxigenic moulds or that would completely inhibit the elaboration of the toxin would be an ideal solution. This approach has been actively under trial at the National Institute of Nutrition, Hyderabad. The main objective is to identify such varieties which support minimal toxin production even under bad conditions of storage. Several varieties of peanuts from different peanut growing regions of the World were screened for their toxin producing potential under laboratory conditions, when infected with a toxigenic strain of *A. flavus*. Of the sixty varieties screened, one variety, US-26 (PI. 246388) was found to be promising since practically no toxin was produced (Rao and Tulpule, 1967). Though agronomically comparably to others, this variety was not commercially popular even in U.S.A. Subsequently, Asiriya Mwitunde (PI. 268893) was reported to be tolerant to aflatoxins (Kulkarni *et al*, 1967). However, these varieties were subsequently found to produce the toxin (Doupnik, 1969). This difference was later ascribed to the toxigenic potential of the fungal isolates used (Nagarajan and Bhat, 1973). In view of the importance of the genetic approach, further attempts are in progress to identify promising varieties of peanuts and maize supporting minimal aflatoxin production. More than 50 varieties of peanuts and maize grown in India are being screened and the few promising varieties that have emerged from this trial are being intensively investigated further (Nagarajan, Bhat and Tulpule, 1974a). Similar trials have been made on soya beans, sunflower seeds and maize varieties (Bhat *et al*, 1974; Nagarajan and Bhat, 1972; Nagarajan *et al*, 1973b, 1974b) are in progress. Recent studies indicate that less amounts of aflatoxins are produced in some varieties of maize, although the amount of fungal growth in grains was not reduced (Priyadarshini and Tulpule, 1978).

5.3 ERGOT CONTAMINATION:

Two general approaches towards prevention of ergot contamination in Pearl millet have been suggested. The first one is to suitably alter agricultural practices in terms of adjusting the cultivating season of the crop in order to reduce the risk of ergot contamination. The second approach is that of decontamination of ergoty grains through floatation in 10-15 per cent salt solution. The ergoty grains floating to the top could be easily removed. This method is already being practised in the belts where Pearl millet is used as staple. Suitable methods of mechanical sieving to remove ergoty grains have also been suggested. Evolving varieties that are resistant to ergot appear to be the ideal solution. Several lines which are resistant to ergot have been evolved by the plant breeders.



Fig. 8. Toxicity of ergot of pearl millet alkaloid toxicity in monkey.
Fig. 9. A woman affected with aflatoxicosis.
Fig. 10. Separation of sound ground-nut kernels by handpicking
(Courtesy : Dr. V. Sreenivasamurthy, CFTRI, Mysore)

6. CENTRES FOR STUDY OF MYCOTOXINS

Two centres of fairly long-standing with adequate infrastructure in terms of equipment and expertise have been the Central Food Technological Research Institute, Mysore and National Institute of Nutrition, Hyderabad. However, in recent years there has been great interest in the field of mycotoxins and their possible health hazards to live-stock and man. It is therefore, obvious that several other centres have also been engaged in research on various aspects of mycotoxins. The aspects of research have been restricted by limitations of facilities available. The major centres currently interested in research in mycotoxins are indicated below:-

<u>Centre</u>	<u>Type of work and facilities</u>
1. Central Food Technological Research Institute, Mysore-13.	All mycotoxins, screening, analytical; epidemiology, detoxification, Training programme.
2. National Institute of Nutrition, Hyderabad-7.	All mycotoxins, screening, analytical, epidemiological; genetic approach for prevention, clinical, biological, chemical, ad-hoc training programmes.
3. Vallabhai Patel Chest Institute, University of Delhi, New Delhi-7.	Aflatoxins - Academic, Experimental; screening, analytical chemical and biological testing.
4. Central Drug Research Institute, Lucknow; and Industrial Toxicology Research Centre, Lucknow.	Aflatoxins Screening, Analytical.
5. Deptt. of Plant Pathology, Punjab Agric. University, Ludhiana (Punjab).	Aflatoxins Screening, analytical.

<u>Centre</u>	<u>Type of work and facilities</u>
6. College of Veterinary and Animal Sciences, Mannuthy (Kerala State).	Aflatoxicosis in live-stock.
7. Madras Veterinary College, Madras-7.	Aflatoxicosis in farm animals, poultry.
8. College of Veterinary Science and Animal Husbandry, J.N.K. Agric. University, Jabalpur (M.P.)	Aflatoxins - Screening, analytical.
9. University Biochemistry Laboratories, Madras-25.	Aflatoxins, <i>Penicillium</i> Toxins - Analytical, experimental.
10. Department of Gastro-enterology and Pathology, All India Institute of Medical Sciences, New Delhi-16.	Pathological aspects of aflatoxin injury, epidemiology.
11. Department of Veterinary Pathology, Haryana Agricultural University, Hissar.	<i>Fusarium</i> toxicosis in animals.

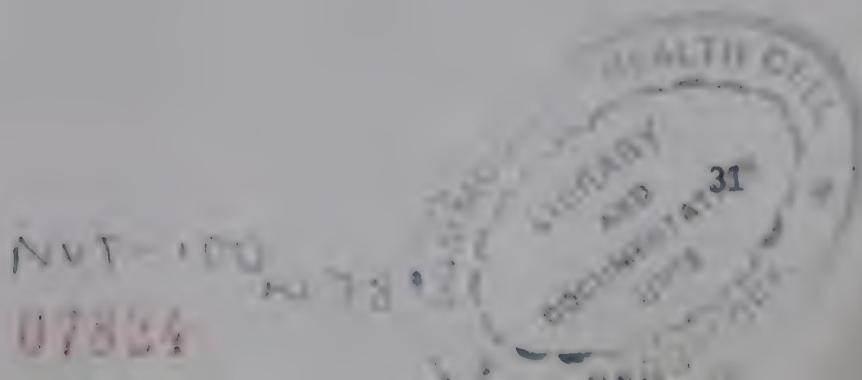
7. NATIONAL REGULATIONS FOR TOLERANCES

7.1 AFLATOXIN LIMITS:

Although there is as yet no national regulation for aflatoxin limits, unofficial limits have been in vogue for quite some time. The unofficial limits fixed for human foods (as finished products or primary foods) have been set at 30 ppb as per the guidelines of the protein Advisory Group (Anonymous, 1970). The Indian Standards Institute, New Delhi has laid down specifications for limits of aflatoxin in expeller processed and solvent extracted edible grade groundnut flour at 120 ppb (Anonymous, 1968). The Central Committee for Food Standards (of the Directorate of Health Services, Ministry of Health, Government of India), has generally recommended a safe limit of 30 ppb, as per guidelines of the PAG. There appears to be as yet no regular safe limit fixation for animal feeds in India. Countries exporting deoiled groundnut cake have their quality control machinery and appear to have fixed a limit of 60-120 ppb. The other organisations involved in the utilisation of deoiled groundnut meal (edible grade) for incorporation into protein-rich food supplements as "balahar" for children (Food and Nutrition Board, Department of Food, Ministry of Food & Agriculture, Food Corporation of India) have generally followed the PAG guideline of 30 ppb as the safe limit.

7.2 ERGOT CONTAMINATION:

A tentative and arbitrary safe limit of 0.05 per cent of ergoty grains in pearl millet has been fixed. This limit is in operation in the procurement campaign launched by the Food Corporation of India (S.V. Pingle, Personal Communication). The Central Committee for Food Standards (Ministry of Health, Government of India) has also tentatively fixed 0.05 per cent as safe limit for ergot contamination.



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8. NEED FOR A NATIONAL PROGRAMME OF SURVEILLANCE FOR MYCOTOXINS IN FOODS AND FEEDS

At the national level, among authorities concerned, there is considerable awareness of the problem of mycotoxins in foods and feeds and their relation to health of live-stock and man. Increased morbidity in poultry farms, dairy farms and the recent episodes of toxic hepatitis in human population in Western India region have caused alarm and awareness of the seriousness of the aflatoxin contamination problem. The Indian Council of Agricultural Research, the Indian Council of Medical Research, the Council of Scientific and Industrial Research and the Food Corporation of India have all been concerned about the problem. However, the current awareness has not as yet resulted in taking concrete steps in the shape of institution of a permanent machinery for monitoring surveillance and control of the problem. This is perhaps immediately necessary in regard to animal feeds and foods meant for human consumption. Such a machinery must necessarily devote attention to monitoring and surveillance of fungal contamination in agricultural commodities; monitoring and surveillance of diseases in live - stock and man arising from mycotoxins in foods and also formulate appropriate measures for control.

In the context of developing a national programme of monitoring and surveillance of mycotoxins, it is appropriate to indicate a few recent developments which are in the right direction. In 1975, a national symposium on food toxins organised by the Nutrition Society of India (Hyderabad) after reviewing the work on some aspects of aflatoxins and ergot toxins made the following recommendations (Anonymous, 1975c).

(a) ORGANISATIONAL MACHINERY:

A suitable machinery for monitoring the incidence of aflatoxins under different conditions of storage should be developed in each state. There has also to be an organised machinery for collecting information on epidemics of unknown etiology (both in man and live-stock) likely to be due to mycotoxins and transmit

the information to the appropriate expertise and institutions for necessary investigations.

(b) BASIC RESEARCH:

Mycotoxins, other than aflatoxin, particularly *Fusarium* toxins and their relevance to health hazards in live-stock and man need detailed studies. From the point of prevention, varietal differences in sensitivity to fungal invasion and toxin elaboration needs to be studied further in peanuts and maize. The biochemical basis for such variations and resistance needs elucidation.

(c) APPLIED RESEARCH:

The effect of different storage methods, both conventional as well as potential like irradiation on susceptibility to fungal invasion and the production of toxins should be studied.

(d) EXTENSION:

There is lot of scope for extension work in educating farmers on better methods of drying, better methods of post-harvest storage and use of chemicals or other conventionally successful methods to prevent fungal contamination.

(e) GAPS REQUIRING IMMEDIATE ATTENTION:

In spite of the available knowledge and the frequent reports of health hazards due to mycotoxins, it has not been possible to assess correctly the seriousness of the problem in terms of the extent of their influence on health since there is no machinery at present undertaking studies of this nature. There are a number of diseases of undetermined etiology but which on retrospective analysis would appear to have characteristics of mycotoxicosis through the food chain. The main reasons for this appears to that there is no proper channel through which outbreaks of diseases of unrecognised etiology are brought to the immediate attention of the scientists in the field. There is therefore a need for a continuous surveillance of population/live-stock exposed to contaminated foods and screening of foods for contamination. Such monitoring should co-ordinate the screening programme with toxicological evaluation and epidemiological studies for establishing the desired link. It is known that aflatoxin crosses the mammary glands and is secreted into milk. The ingestion of such contaminated milk and the consequences of such intakes for long periods by infants and children have to be investigated. This has

to be taken note of in the monitoring systems for aflatoxin.

The other important development in the recent period, in the field of aflatoxin, is the organisation of a workshop on aflatoxin in oil seeds and other products. This workshop held at the Central Food Technological Research Institute, Mysore in March, 1976, was organised by the FAO/SIDA/TF and attended by technical experts of different disciplines, drawn from India, Indonesia, Philippines, Senegal, Ceylon, Sudan, Thailand, Turkey, Uganda and Tanzania. The purpose of the workshop was to provide training in methodological approaches in detection, analysis, control and prevention of aflatoxin, to food analysts and food inspection staff from the main producing and exporting countries of oil seed products (Anonymous, 1976n). This workshop assumes a lot of significance as a step in the right direction since it was attended by participants drawn from different countries where aflatoxin problem in agricultural commodities continues to be a challenge in terms of economy and health hazard.

In the light of these developments, it would perhaps be appropriate to suggest that the time is ripe enough to initiate a co-ordinated national programme of monitoring, surveillance and control for mycotoxins. Such co-ordinated national programme is envisaged to consist of a three-tier system of operational investigation and action programme. These are (a) commodity-tier dealing with screening of agricultural commodities from different regions and assessing the toxigenic potential of fungal contamination; (b) animal-health-tier dealing with quality control of all animal feeds and epidemiological and other associated studies of disease in live-stock relatable to mycotoxin origin; and (c) human health-tier dealing with quality control of foods for human consumption and epidemiological and other associated investigations on human health hazards relatable to mycotoxic origin.

Such a three-tier system and its co-ordinated programme should have a nucleus at each state level. The important elements to be taken care of are surveillance, tolerance limits, health aspects, prevention and control. The currently existing Indian Council of Agricultural Research - Indian Council of Medical Research Joint Panel on Food,, Nutrition and Health as also that of the Indian Council of Agricultural Research Panel on post-harvest technology could be strengthened to initiate the co-ordinated programme at the National level. The currently available data suggests that the high-risk belts include Kerala, Coastal Mysore, Thanjavur (Tamil Nadu), Coastal Andhra, Western India belt, Punjab-Haryana, Gangetic plains and North-Eastern region. The co-ordinated national programme could immediately attend to these

regions. Since Central Organisations such as Indian Council of Agricultural Research, Indian Council of Medical Research, Council of Scientific and Industrial Research and Department of Science and Technology are already concerned about the problems and their implications in terms of economy conserving valuable food resources and health aspects in live-stock and man, the question of finding financial resources for the co-ordinated national programme is not insurmountable. As an immediate beginning, it is suggested that a national workshop could be organised involving all the disciplines and expertise available for drawing up time-bound action programme.

It is appropriate to appreciate the role of international organisations like F.A.O. and W.H.O. in regard to their concern about consumer protection against food contaminants. The recent "Expert Consultation on the Joint F.A.O./W.H.O. Food Contamination Monitoring Programme" of a global magnitude provides ample evidence of their concern (Anonymous, 1975b). As part of such a world-wide programme, the F.A.O./W.H.O. could perhaps be of great assistance in organising a workshop in India, for the South-East Asia region where fungal contamination is still a major problem. The convening of such a workshop will provide a good forum for the exchange of experience from different countries in the region, on the modus operandi of approaches for assessing the extent of problem, monitoring and control operations.

It is fervently hoped that the objectives of this compilation of this country report will be more than achieved and served, if it could help to motivate those concerned for initiating a co-ordinated programme for surveillance of the problem in all its multifaceted aspects for the welfare of live-stock and man.

9. SUMMARY

1. Several environmental factors present in a tropical country like India and widely varying but unsatisfactory post-harvest storage practices appear to be conducive to the development of fungi of different species some of which are toxigenic.
2. The prevalence of fungal contamination, the nature of the fungal species involved and their toxigenic potential have been investigated on several agricultural commodities. The most widely investigated commodities include groundnuts, groundnut cakes, cereals, millets and to some extent pulses and animal feeds.
3. There appears to be overwhelming evidence to suggest that *A. flavus* contamination and the presence of aflatoxin is a serious problem in most agricultural commodities. Most of the isolates of *A. flavus* screened from agricultural commodities proved to be toxigenic. Among the other species of fungi, those that are more commonly encountered are *Penicillium*, *Fusarium* and *Claviceps*. Among these, except for *Claviceps* species, the toxigenicity of the others appear to be relatively less studied.
4. Mycotoxicoses in animals, particularly those of economic importance such as dairy cattle and poultry have been encountered on many occasions. Sporadic outbreaks of diseases due to mycotoxins have been encountered in other animals as rabbits, pigs and dogs. Most of these out-breaks have been ascribable to aflatoxins. The problem of mycotoxins entering the food-chain through dairy and poultry products (mycotoxins contamination by proxy) has not been adequately investigated.
5. Aflatoxicosis has been studied extensively in experimental animals using rat and the primate models. The acute and chronic toxic effects of aflatoxin injury have been investigated. The demonstration of acute liver injury and carcinogenic effects in monkeys has been of great significance in our attempts to extrapolate the possible health hazards to man. The increased susceptibility of experimental animals to acute liver damage due to aflatoxin in conditions of nutritional deprivation, specially

protein and vitamin A is again of significance in view of the fact that human population in large parts of the country are concurrently exposed to nutritional stress and fungal contamination of agricultural commodities.

6. Variation in susceptibility to mycotoxicoses, particularly aflatoxicoses in different species of animals has brought out interesting observations. The differences in susceptibility of aflatoxicoses between cows and buffaloes is of great significance since buffaloes which are more susceptible are the major source of milch cattle in large parts of India.

7. Mycotoxicoses in human has perhaps not received as much attention as they deserve. However, there have been well documented evidence of mycotoxicoses like ergotism, mouldy ragi poisoning, kodo millet poisoning and polyuria. In most of these outbreaks, the ill effects on health appeared to be transitory. An outbreak of acute hepatitis in parts of Western India, due to aflatoxin contamination in maize can be considered as the first evidence of direct implication of acute aflatoxicosis in man encountered in epidemic proportions. This experience has focussed attention on the possible risk of aflatoxin contamination in staples.

8. Approaches for prevention and control of mycoflora contamination in general have been under trial only on a limited scale. Several attempts to control fungal growth in agricultural commodities have been made through the use of fungicides, gaseous steriliants and other fungistatic agents. In the field of aflatoxin contamination, processing methods for detoxification of de-oiled groundnut meal has received great attention. Genetic approach to identify varieties resistant to *A. flavus* invasion and toxin elaboration has been under trial and if successful, will prove to be of practical application at the farmer's level. The question of control of ergot infestation appears to be feasible through appropriate changes in agricultural practices as altering the sowing season or through simple methods of decontamination of ergot contaminated bajra. It is increasingly being realised that as a general measure of prevention of fungal attack, great emphasis is needed in developing better methods of quick drying and improved storage structures. Greater inputs in these approaches will be rewarding.

9. Currently, there are atleast ten centres in India, having good research infrastructure and expertise for carrying out research on all aspects of mycotoxins. The multidisciplinary character of these centres needs further reinforcement to be

fruitful for developing them as regional centres of excellence and provide a national base for mycotoxic research.

10. National regulations for tolerance limits to most of the mycotoxins have not yet been made. However, in regard to aflatoxin, there has been an unofficial limit set at 30 ppb, on the basis of the recommendations of the Protein Advisory Group of the United Nations. There appears to be no official regulatory limit for animal feeds in India. Countries importing groundnut cake from this country appear to have fixed a limit of 60-120 ppb. In regard to ergot contamination, an arbitrary safe limit of 0.05 per cent ergoty grains in millets has been in operation.

11. There is enough evidence that mycotoxin contamination in foods and feeds is increasingly being encountered and is causing enough damage to the health of man and live-stock besides resulting in economic loss in terms of wasted food materials. There is also an awareness of the problem at a national level. It is therefore appropriate that a national programme of surveillance for mycotoxins in foods and feeds be initiated. It is suggested that such a national programme should constitute a well coordinated three-tier system for research and action programme. The three-tiers include commodity-tier, animal health-tier and human-health tier. The Indian Council of Agriculture Research, Indian Council of Medical Research, Council of Scientific and Industrial Research the Department of Science and Technology, the Food Corporation of India and the Indian Standards Institution could jointly sponsor such a national programme.

12. International agencies such as F.A.O. and W.H.O. could be of great help in assisting national programmes of surveillance in countries of the South East Asia region, where mycotoxin problems are still a challenge to the health of live-stock and man.

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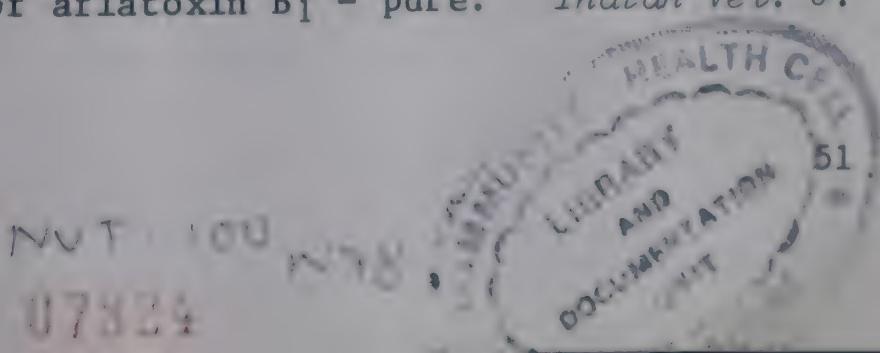
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